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Predominant pathogenic role of tumor necrosis factor in experimental colitis in mice

Antibodies to tumor necrosis factor (TNF)-α have been recently proposed as effective treatment for patients with Crohn's disease. Here, we analyze the functional role of TNF-α in a mouse model of chronic intestinal inflammation induced by the hapten reagent 2,4,6,-trinitrobenzene sulfonic acid (TNBS) that mimics some characteristics of Crohn's disease in humans. Macrophage-enriched lamina propria (LP) mononuclear cells from mice with TNBS-induced colitis produced 10–30-fold higher levels of TNF-α mRNA and protein than cells from control mice. When mice with chronic colitis were treated by intraperitoneal injection of antibodies to TNF-α, an improvement of both the clinical and histopathologic signs of disease was found. Isolated macrophage-enriched LP cells from anti-TNF-α-treated mice produced strikingly less pro-inflammatory cytokines such as interleukin (IL)-1 and IL-6 in cell culture. The predominant role of TNF-α in the mouse TNBS-induced colitis model was further underlined by the finding that striking colonic inflammation and lethal pancolitis was induced in TNF-α-transgenic mice upon TNBS treatment. Conversely, no significant TNBS-induced colitis could be induced in mice in which the TNF-α gene had been inactivated by homologous recombination. Complementation of TNF-α function in $TNF^{-/-}$ mice by the expression of a mouse TNF-α transgene was sufficient to reverse this effect. Taken together, the data provide direct evidence for a predominant role of TNF-α in a mouse model of chronic intestinal inflammation and encourage further clinical trials with antibodies to TNF-α for the treatment of patients with Crohn's disease.

1 Introduction

The focus of much recent clinical and basic immunological research has been the regulation of cytokine expression in chronic intestinal inflammation [1–3]. These data showed that changes in cytokine production by lymphocytes and macrophages are very likely to play a major role in the pathogenesis of inflammatory bowel diseases (IBD), which include Crohn's disease (CD) and ulcerative colitis (UC). These data also provided reason to believe that mucosal T cell responses in CD are characteristically associated with an IFN-γ cytokine response, whereas those in UC are associated with an IL-5 response possibly caused by differential production of IL-12 by lamina propria (LP) mononuclear cells between CD and UC [4]. Furthermore, there is an increased production of pro-inflammatory cytokines by LP mononuclear cells in both diseases, suggesting that changes in the balance between pro- and anti-inflammatory cytokines may be crucial for development of chronic intestinal inflammation. Recent studies by van Dullemen et al. [5] have shown that regulation of cytokine levels in patients with IBD may also be a novel approach for treatment of these diseases. They described successful treatment of established intestinal inflammation in patients with Crohn's disease by a single intravenous infusion of antibodies to TNF-α, suggesting a key role for this cytokine in the pathogenesis of chronic intestinal inflammation.

TNF-α is also a member of the TNF family of proteins that comprises lymphotoxin-α and lymphotoxin-β [6–10]. TNF-α is involved in the regulation of many infectious, inflammatory, and autoimmune processes, such as cachexia and wasting disease in mice, antibacterial host defense, contact hypersensitivity reactions, LPS-induced endotoxic shock, induction of fever, and stimulation of production of several acute-phase proteins. It is mainly produced by T cells and macrophages after stimulation with various microbial agents, such as LPS. Human TNF-α is initially synthesized as a 26-kDa precursor containing an N-terminal presequence which is removed prior to or during secretion, resulting in a mature peptide of 17-kDa which may form homotrimeric secreted molecules that can bind to two distinct cell surface receptors, designated p55 and p75 TNFR [11–16]. TNF receptors are part of a family of membrane proteins which include the nerve growth factor receptor, Fas antigen, CD27, CD30, CD40, Ox40, 4-1BB, and a receptor for the lymphotoxin-α/β heterodimer. Receptors are aggregated by the TNF trimer into clusters of two or three molecules of either the 55- or 75-kDa TNFR [11–13]. Most cells express both TNF receptors, which appear to mediate different biological functions. Whereas p75 TNFR signaling has been mainly implicated in lymphocyte proliferation, the 55-kDa receptors regulate TNF-mediated cytotoxicity, apoptosis, antiviral activity, fibroblast proliferation, and NF-κB activation. In the pres-

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Abbreviations: **TNBS:** 2,4,6,-Trinitrobenzene sulfonic acid **CD:** Crohn's disease **UC:** Ulcerative colitis **LP:** Lamina propria **IBD:** Inflammatory bowel disease **LPMC:** Lamina propria mononuclear cells

Key words: Tumor necrosis factor-α / Inflammatory bowel disease / Crohn's disease / Inflammation

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ent study, we demonstrate a predominant pathogenic role for TNF-α in a mouse model of experimental colitis. These data encourage further clinical trials employing anti-TNF strategies in patients with IBD.

2 Materials and methods

2.1 Induction of colitis

Specific pathogen-free 2-4-months-old female SJL/J mice were obtained from the National Cancer Institute (NCI, Bethesda, MD). The generation and characterization of TNF-α knockout mice has been described [17]. In brief, the targeting vector contained a thymidine kinase (tk) gene under the control of the MC1 promoter upstream of the MC1neopA vector cassette with homologous flanking sequences. Tg197- and Tg86-transgenic mice expressing human wild-type and mutant mouse transmembrane TNF protein, respectively, have been described [17, 18]. 2,4,6,-Trinitrobenzene sulfonic acid (TNBS) colitis in mice was induced as described [19, 20].

2.2 Grading of histologic changes

Tissues were removed from the TNBS-treated mice at the indicated time points. Cryosections (7 μm) were made and stained with hematoxylin and eosin. The degree of inflammation on microscopic cross-sections of the colon was graded semiquantitatively from 0 to 5 according to previously described criteria [20]. Grading was done in a blinded fashion by the same pathologist.

2.3 Electron microscopy

For transmission electron microscopy, specimens were fixed in 1 % osmium tetroxide in 0.2 M sodium cacodylate buffer (pH 7.4) at 4 °C. Dehydration was performed with graded ethanols (50, 70, 80, 90, 95, 98, and 100 %), and samples were embedded in Spurr's Epon. Ultrathin sections 50–90 nm thick were contrasted with uranyl acetate/lead citrate and analyzed with an EM10 Zeiss microscope.

2.4 Cell isolation and purification of macrophage-enriched lamina propria T cells

Lamina propria lymphocytes were isolated from freshly obtained colonic specimens using a modification of the technique established by van der Heijden and Stok, as described [21]. In brief, the resected colon was washed thoroughly in Hank's balanced salt solution free of calcium and magnesium and Peyer's patches were removed. The colons were then opened longitudinally, cut into 0.5 cm pieces and incubated twice in Ca- and Mg-free balanced salt solution containing 0.37 mg/ml EDTA and 0.145 mg/ml DTT in a shaking incubator at 37 °C for 15 min to remove epithelial cells and intraepithelial lymphocytes. After decanting the supernatant, the resultant colonic tissue was incubated for 90 min in RPMI 1640 containing 10 % heat inactivated fetal calf serum, 25 mM Hepes, 400 U/ml collagenase D (Boehringer Mannheim, Indianapolis, IN) and 0.1 mg/ml DNase I (Boehringer Mannheim) in a shaking incubator at 37 °C. The supernatant was collected by filtration straining through a 40 μm mesh nylon cell strainer (Falcon, Becton Dickinson Labware, NJ). LP cells were then layered on a 40 %/100 % Percoll step gradient (Pharmacia, Uppsala, Sweden) and centrifuged at 1500 rpm for 20 min at 4 °C. Cell populations isolated at the 40–100 % interface were further enriched for macrophages as described [19].

2.5 Cell culture of LP cells

LP cells were cultured in complete medium consisting of RPMI 1640 (Whittaker, Walkersville, MD) supplemented with 3 mM L-glutamine, 10 mM Hepes, 10 μg/ml gentamycin (Whittaker), 100 U/ml penicillin and 100 μg/ml streptomycin (Whittaker), 0.05 mM 2-ME (Sigma, St. Louis, MO) and 10 % heat-inactivated fetal calf serum. Cells were resuspended in complete RPMI and cultured in complete medium with or without 40 μg/ml LPS (*E. coli* strain B4:0111; obtained from Sigma) and 10 μg/ml PHA (obtained from Sigma).

2.6 PCR analysis

Total cellular RNA from LP cells was isolated by the acid guanidinium thiocyanate/phenol/chloroform extraction method. Reverse transcriptase (RT) reaction of heat denatured total RNA with M-MLV RT was performed according to the manufacturer's protocol (Gibco BRL, Gaithersburg, MD). From the obtained cDNA, the TNF coding region was amplified by PCR (denaturation 94 °C for 3 min; 94 °C 1 min, 55 °C 2 min, 72 °C 3 min for 30 cycles; and a final extension at 72 °C for 10 min) using specific primers derived from published sequence data [19].

2.7 Northern hybridization

LP mononuclear cells were isolated as described above and total cellular RNA from these cells was isolated by the acid guanidinium thiocyanate/phenol/chloroform extraction method. RNA (10 μg) was separated on agarose gels that were blotted onto 0.2-μm pore nitrocellulose membranes. Northern blots were hybridized to specific cDNA probes corresponding to TNF-α and β-actin sequences that were generated by RT-PCR amplification from spleen B cell cDNA.

2.8 Western blot analysis

For Western blotting, 50 μg proteins from macrophage were blotted to a 0.45-μm pore nitrocellulose membrane followed by incubation with rat anti-TNF-α antibody. After incubation with alkaline phosphatase-labeled goat anti-rat antibody, detection was performed using alkaline phosphatase color substrate (obtained from Promega, Madison, WI) as previously described [19].

2.9 Cytokine assays

To measure cytokine production, 2×10^5 LP cells were cultured in 1 ml complete medium, and LPS and PHA were added as specified above. Cultures were incubated at 37 °C in a humidified incubator containing 6 % CO_2 in air. After 24 h, culture supernatants were removed and assayed for cytokine concentration.

Cytokine concentrations were determined by specific ELISA. Monoclonal rat anti-mouse cytokine antibodies were diluted at 2 μg/ml in 2 mM carbonate buffer, pH 9.6. Antibody solution (50 μl) per microwell was then aliquoted into Immulon-4 96-well microtiter plates (Dynatech Laboratories Inc., Chantilly, VA). Plates were incubated at 4 °C overnight, washed twice with PBS/Tween 20, and blocked for 2 h with 3 % BSA in PBS. At this point, 100 μl sample or standards of recombinant mouse cytokines (Genzyme, Boston, MA) were added to wells and allowed to incubate at room temperature for 2 h. Plates were then washed four times, after which 100 μl biotin-labeled rat anti-mouse cytokine antibody (2 μg/ml in PBS + 3 % BSA) was added to each well and incubated at room temperature for 45 min. Plates were then washed an additional six times, after which 100 μl 1: 1000-diluted solution of horseradish peroxidase-labeled streptavidin (Zymed, San Francisco, CA) in PBS with 3 % BSA was added to each well. Plates were then incubated for 30 min at room temperature and washed twelve times, after which 100 μl o-phenylenediamine dihydrochloride (15 mg tablet/15 ml phosphate-citrate buffer with 0.01 % H_2O_2, obtained from Sigma) was added to each well. Absorbance was measured on a Dynatech MR 5000 ELISA reader at a wavelength of 490 nm.

2.10 Treatment with antibodies to TNF

Neutralizing rat anti-mouse TNF mAb were kindly donated by R. Seder (National Institutes of Health). Rat control IgG was obtained from Jackson Immuno-Research (West Grove, PA). Rat anti-mouse TNF antibodies or rat control IgG (1 mg) were administered intraperitoneally into mice pretreated with TNBS twice a week.

2.11 Elispot assay for TNF

LP cells (1×10^5) were incubated for 1 day in 24-well plates in the presence of LPS + PMA. At the end of the incubation period, cells were washed three times and incubated for 12 h in 96-well plates coated with 2 μg/ml rat anti-mouse TNF (Pharmingen) in coating buffer (0.1 M $NaHCO_3$, pH 8.2) overnight at 4 °C, washed twice in PBS/Tween, blocked with PBS containing 25 % FCS for 2 h at 37 °C, and washed four times with PBS/Tween. After 12 h, plates were washed four times in PBS/Tween and 2 μg/ml biotinylated secondary antibody (Pharmingen) was added and incubated overnight at 4 °C. Plates were washed 6 times in PBS/Tween and streptavidin labeled with alkaline phosphatase (1:1000 dilution; Zymed) was added for 30 min at 37 °C. Plates were washed again eight times in PBS/Tween and the alkaline phosphatase substrate (Western blue stabilized substrate for alkaline phosphatase, Promega, Madison, WI) in 1 % agarose was added. The color reaction was allowed to proceed for 24 h before spots were counted and photographed.

3 Results

3.1 Antibodies to TNF-α ameliorate established TNBS-induced colitis in SJL/J mice

Treatment with antibodies to TNF-α has been recently suggested as an effective therapy for patients with Crohn's disease. In an initial series of studies, we determined TNF-α

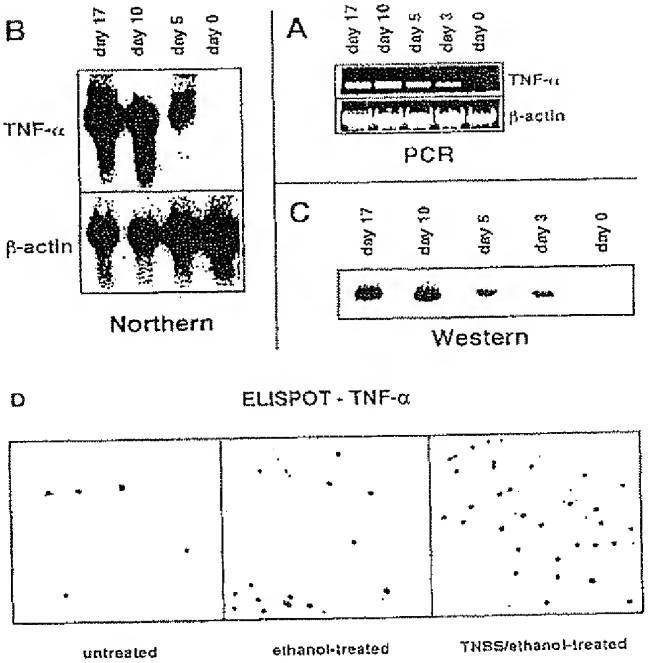
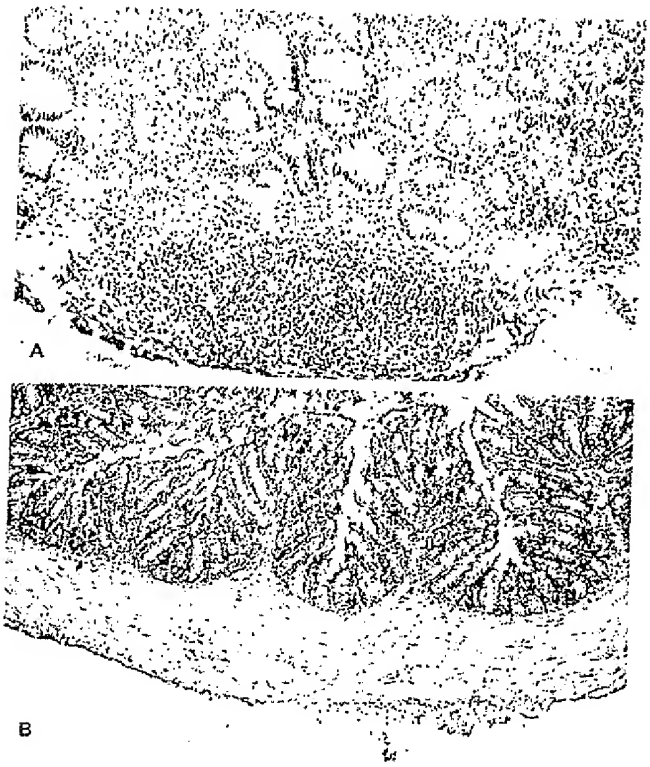


Figure 1. TNF-α mRNA and protein levels are increased in TNBS-induced colitis. (A) PCR analysis for TNF-α and β-actin transcripts of RNA from macrophage-enriched LPMC isolated from the colon of TNBS-treated mice at indicated time points. LPMC were isolated as described in Sect. 2.4 and further enriched for macrophages by negative selection techniques. RNA from these cells was extracted and subjected to exponential PCR amplification using specific primers as specified in Sect. 2.6. (B) Northern analysis for TNF-α and β-actin transcripts of RNA from macrophage-enriched LPMC. RNA samples were analyzed by agarose gels that were blotted onto nitrocellulose membranes. Northern blots were hybridized to specific cDNA probes corresponding to TNF-α and β-actin sequences. (C) Western blotting studies using cellular proteins from macrophage-enriched LPMC isolated from TNBS-treated mice at indicated time points. For Western blotting, 50 μg nuclear proteins from macrophage-enriched LPMC were blotted to a 0.45 μm nitrocellulose membrane and probed with a rat anti-mouse TNF-α antibody. Detection was performed after incubation with an alkaline phosphatase-labeled goat anti-rabbit antibody (Sect. 2.8). (D) ELISPOT assay for TNF-α secretion. LP cells from TNBS- or ethanol-treated, or untreated SJL/J mice were isolated and cultured as specified in Sect. 2.4. Detection of TNF-α was then performed using specific antibodies (Sect. 2.11). A high number of ELISPOTS per high power field was seen in the TNBS-treated group compared to the control group.



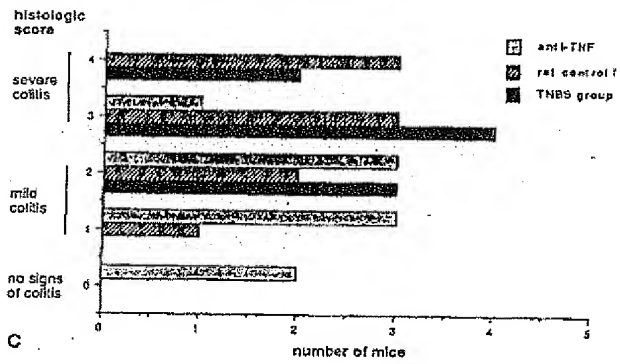


Figure 2. Anti-TNF-α treatment ameliorates established TNB induced colitis. (A) The photomicrograph shows a hematoxyli eosin-stained cross-section (× 100) of a colon of a SJL/J mou with TNBS-induced colitis after treatment with rat control IgG day 10 after initial administration. There was a severe colitis. (E Photomicrograph of hematoxylin/eosin-stained cross sectio (× 50) of a colon of a SJL/J mouse with TNBS-induced colit after treatment with anti-TNF-α antibodies at the same tim point. There was a clear reduction in the inflammatory changes c the colon. (C) Histologic grading of colon sections from SJL/ mice with TNBS-induced colitis after treatment with antibodie to TNF-α. Colon specimens were taken 10 days after administra tion of anti-TNF or rat IgG, and the magnitude of inflammator changes in the colons was analyzed on hematoxylin/eosin-staine colon cross-sections. Data were pooled from two independen experiments.

expression levels in a mouse model of chronic intestinal inflammation induced by the haptenizing reagent TNBS that mimics some characteristics of Crohn's disease in humans [19–20]. Accordingly, we assayed the ability of macrophage-enriched LP mononuclear cells (LPMC) from TNBS-treated mice to produce TNF-α. We found that these cells produced high levels of TNF-α mRNA and protein (Fig. 1, Table 1), consistent with previous reports on increased TNF-α production by LP cells in patients with Crohn's disease. The observed increase in TNF-α secretion by LP cells from TNBS-treated mice could have been due to increased TNF-α secretion per cell or to secretion by a larger portion of the cells. To investigate this question, we performed ELISPOT assays for TNF-α secretion on LP cells. As shown in Fig. 1D, a dramatic increase in the average number of ELISPOTS was found using LP cells from TNBS-treated mice compared to LP cells from control ethanol-treated mice. The size of the ELISPOTS, however, was similar in both groups, indicating that the increase in TNF-α secretion by LP cells from TNBS-treated mice is mainly due to an increase in the number of TNF-α secreting cells.

We next addressed the functional role of TNF-α in TNBS-induced colitis by treating mice with established colitis with antibodies to TNF-α (Fig. 2). Repeated administration of antibodies to TNF-α ameliorated experimental colitis 10 days after the beginning of treatment, as assessed by weight analysis (data not shown). Histologically, anti-TNF-treated mice showed less severe colitis compared with control rat IgG-treated mice (Fig. 2A–B). This finding was consistent with an improvement of the histological score of colitis in anti-TNF-treated animals (Fig. 2C). Furthermore, we found that macrophage-enriched LP cells from

Table 1. Cytokine secretion by lamina propria macrophages[a)]

	IL-1 (pg/ml)	IL-6 (pg/ml)	TNF-α (U/ml)
Normal	4 ± 0.3	23 ± 3.5	24 ± 5.5
Ethanol	4 ± 0.5	20 ± 1.7	35 ± 7.1
TNBS	66 ± 9.4	211 ± 19.1	355 ± 41.2
TNBS + anti-TNF	7 ± 0.9	18 ± 8.1	1 ± 0.1
TNBS + rat IgG	61 ± 9.1	191 ± 12.2	328 ± 40.1

a) Secretion of IL-1, IL-6, and TNF-α by LP macrophages from normal mice, ethanol-, or TNBS-treated SJL/J mice and from mice with TNBS-induced colitis given anti-TNF-α at day 17. LP macrophages were isolated from normal SJL/J mice and from untreated and treated mice with TNBS-induced colitis at day 17 after administration of TNBS. Supernatants were collected after 24 h and analyzed for cytokine content by specific ELISA.

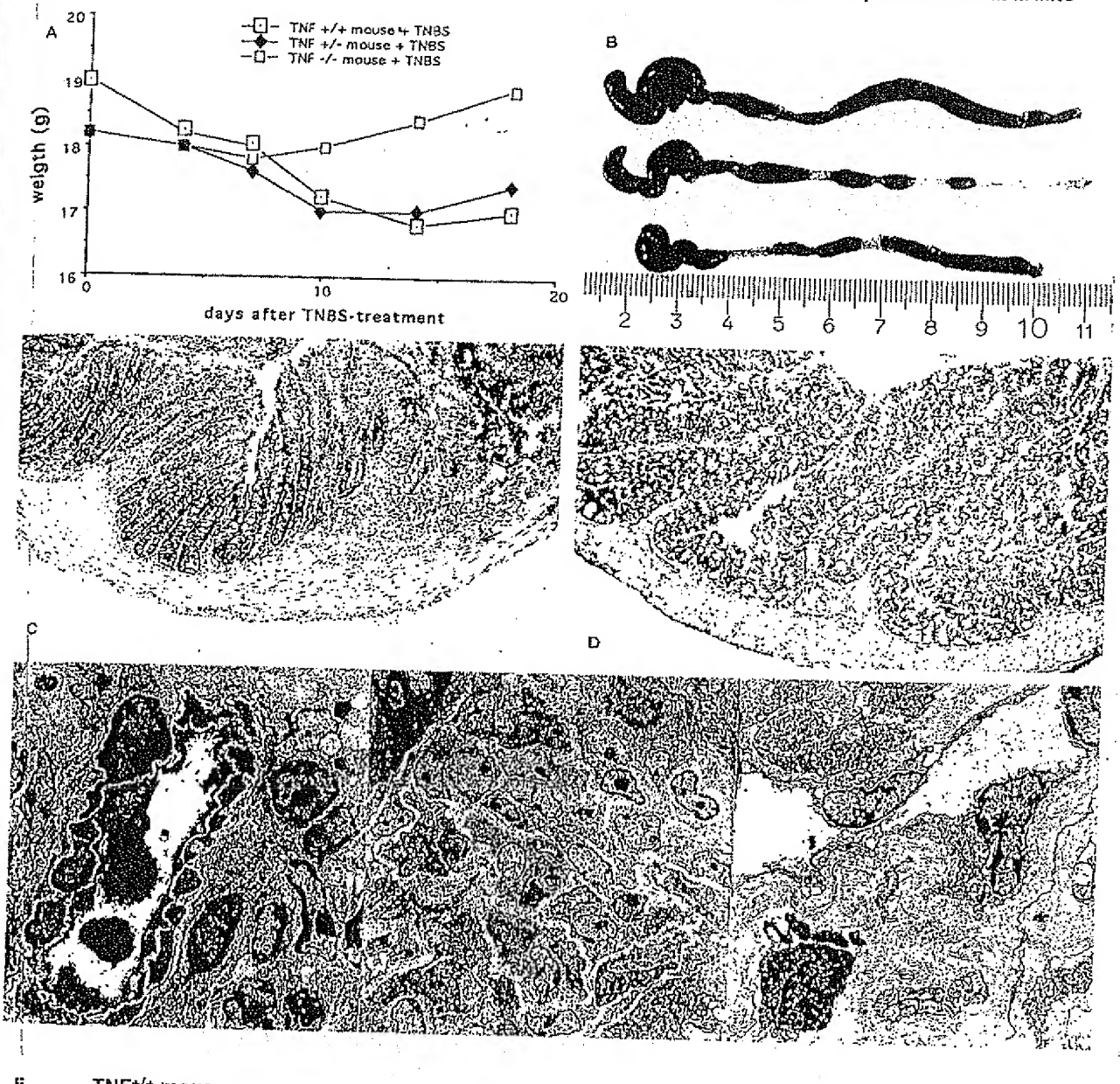


Figure 3. Lack of chronic colitis in TNBS-treated $TNF^{-/-}$ mice. (A) Weight changes in $TNF^{+/+}$, $TNF^{+/-}$ and $TNF^{-/-}$ mice after intrarectal administration of TNBS. Mice in which the TNF-α gene had been inactivated did not show significant weight loss compared to wild-type littermates. One out of two representative experiments is shown. (B) Macroscopic changes of colon in TNBS-treated $TNF^{+/+}$, $TNF^{+/-}$ and $TNF^{-/-}$ mice. Photographs of dissected large intestine of a $TNF^{+/+}$ mouse (top), a $TNF^{-/-}$ mouse (second row), and a $TNF^{+/-}$ mouse are shown. The colons of the TNBS-treated $TNF^{+/+}$ and $TNF^{+/-}$ mice are strikingly thickened. (C–D) Photomicrographs of hematoxylin/eosin-stained cross-section (× 100) of a colon of $TNF^{+/+}$ (C) and $TNF^{-/-}$ (D) mice at day 14 after TNBS treatment are shown. There was no severe colitis in $TNF^{-/-}$ mice. (E) Ultrastructural analysis of the colon of $TNF^{-/-}$ and $TNF^{+/+}$ mice after TNBS treatment: there was a hypervascularization with swelling of endothelial cells (left panel) and severe inflammation in the colon of TNBS-treated $TNF^{+/+}$ mice. In contrast, there were strikingly fewer ultrastructural signs of mucosal inflammation in the colon of TNBS-treated $TNF^{-/-}$ mice (right panel).

Table 2. Cytokine secretion by LP cells in $TNF^{-/-}$ mice and in TNF-α-transgenic mice[a]

	IL-1 (pg/ml)	IL-6 (pg/ml)	TNF-α (U/ml)
Normal	4 ± 0.3	23 ± 3.5	24 ± 5.5
Ethanol	4 ± 0.5	20 ± 1.7	35 ± 7.1
$TNF^{+/+}$ mouse + TNBS	44 ± 3.1	181 ± 11.1	391 ± 32.1
$TNF^{-/-}$ mouse + TNBS	7 ± 0.9	31 ± 3.1	0.3 ± 0.0
TNF wild-type mouse + TNBS	59 ± 4.1	198 ± 12.2	355 ± 41.9
TNF-transgenic mouse + TNBS	72 ± 9.7	253 ± 21.1	> 1500

a) Secretion of IL-1, IL-6, and TNF-α by LP macrophages from $TNF^{-/-}$ mice and TNF-α-transgenic mice at day 10 after treatmen TNBS. Supernatants were collected after 24 h and analyzed for cytokine content by specific ELISA.

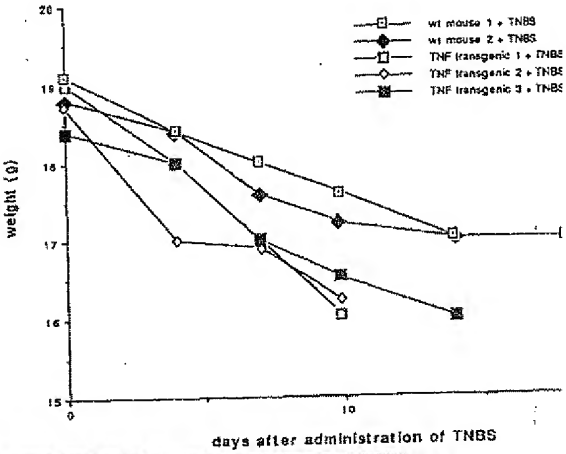
anti-TNF-α-treated mice produced strikingly less pro-inflammatory cytokines such as IL-1 and IL-6 in cell culture (Table 1).

3.2 TNF-α knockout mice do not develop chronic TNBS-induced colitis

To characterize further the functional role of TNF-α in TNBS-induced colitis, we next tried to establish TNBS-induced mucosal inflammation in mice in which the gene for TNF-α had been inactivated by homologous recombination. It was found that $TNF^{-/-}$ mice showed only a transient weight loss upon TNBS treatment, but failed to develop chronic intestinal inflammation (Fig. 3A). Macroscopically, there were no severe signs of colitis in TNBS-treated $TNF^{-/-}$ mice, whereas TNBS-treated control mice showed clear bowel wall thickening (Fig. 3B). Consistently, $TNF^{-/-}$ mice did not show striking histological or ultrastructural signs of colitis (Figs. 3D, 3E). Furthermore, isolated LP cells from these mice failed to produce high amounts of IL-1 and IL-6 (Table 2). In contrast, $TNF^{+/+}$ and $TNF^{+/-}$ mice showed longer-lasting mucosal inflammation, as assessed by weight and histologic analyses, that was accompanied by high-level production of IL-1 and IL-6 by LP cells from these mice (Figs. 3D and 3E, Table 2). Complementation of TNF-α function in $TNF^{-/-}$ mice by cross-breeding with TgA86 mice that express a mutant mouse transmembrane TNF-α transgene [17] was sufficient to reverse these effects, since the resulting mice showed striking mucosal inflammation upon TNBS exposure (data not shown). Taken together, these data suggested a predominant role for TNF-α in maintenance of chronic intestinal inflammation. Moreover, they indicate that transmembrane TNF, at least when overexpressed, is sufficient to induce inflammatory pathology in the gut.

3.3 TNF-α-transgenic mice develop lethal pancolitis upon TNBS exposure

In subsequent studies, we analyzed the role of TNF-α in TNBS-induced colitis by the use of TNF-α-transgenic mice. Here, we analyzed Tg197 mice that express in their tissues a TNF-α transgene, as described [18]. It is worth noting that human TNF signals only through the p55 TNFR and not through the murine p75 TNFR. When TNF-transgenic Tg197 mice were treated by intrarectal administration of TNBS, a more severe colitis was induced compared to wild-type animals. In fact, several animals died within days after treatment (Fig. 4A). Histologi TNBS-treated TNF-α-transgenic mice showed m pancolitis with infiltrates of granulocytes, macroph and T lymphocytes (Fig. 4B). Furthermore, macrop enriched LP cells from TNBS-treated TgA197 mice duced increased amounts of the pro-inflammatory kines IL-1 and IL-6 in cell culture (Table 2). These suggest that overexpression of TNF *in vivo* has a



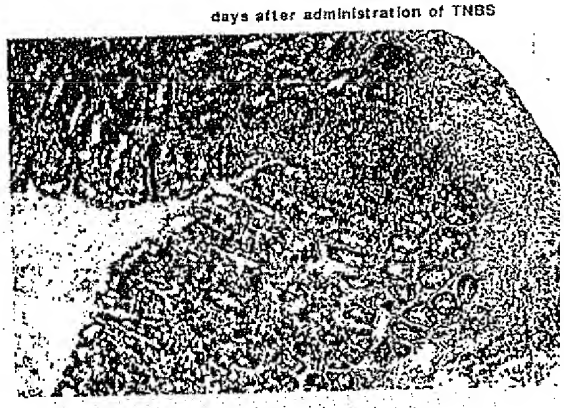


Figure 4. TNBS-treated TNF-α-transgenic mice devel pancolitis. (A) Weight changes of TNF-α-transgenic mice trol mice treated with TNBS in 50% ethanol. Weight c one representative experiment are shown. (B) Photom of hematoxylin/eosin-stained cross section (× 100) of th a TNF-α-transgenic mouse at day 5 after TNBS treatme was a very severe colitis in TNF-α-transgenic mice.

genic role in TNBS-induced colitis and that the p55 TNFR is sufficient to mediate this pathology.

4 Discussion

In the present study, we defined an important pathogenic role for TNF-α in a mouse model of chronic intestinal inflammation induced by the hapten reagent TNBS. First, we demonstrated a striking overexpression of TNF-α in TNBS-induced colitis; second, antibodies to TNF-α ameliorated clinical and histological signs of colitis; third, no chronic colitis could be induced in TNF-α knockout mice; and fourth, we showed that TNF-α-transgenic mice develop lethal pancolitis upon TNBS treatment. Taken together with the increased TNF-α production by LP macrophages in patients with Crohn's disease in humans, these data suggest the potential utility of anti-TNF treatment in patients with this disease.

Recently, various new animal models of chronic intestinal inflammation have been described that will lead to a more detailed understanding of the pathogenic immunological mechanisms underlying mucosal inflammation. These models include rats carrying transgenes for HLA-B27 and β2-microglobulin [22], T cell-reconstituted Tgε26 mice transgenic for the human CD3ε gene [23], and mice in which the genes for IL-2 [24], IL-10 [25], $G\alpha_{i2}$ [26] and the α or β chain of the T cell receptor [27] have been inactivated by homologous recombination. In addition, two Th1-mediated granulomatous colitis models have been established by the adoptive transfer of normal $CD45RB^{hi}$ T cells from BALB/c mice to C.B.-17 *scid* mice [28] and by intrarectal administration of the hapten reagent TNBS in SJL/J mice [20]. Most of these colitis models have been shown to be mediated by $CD4^+$ T cells that produce high amounts of IFN-γ and TNF-α. The high-level production of IFN-γ and Th1 T cell development in these models could be due to the increased production of IL-12, a cytokine produced by macrophages mainly in response to bacterial antigens. The important role of IL-12 in chronic intestinal inflammation is also supported by the finding that antibodies to IL-12 abrogated chronic TNBS-induced colitis [20] and colitis in IL-2- [29] or IL-10- (M. Neurath, unpublished data) -deficient mice.

The mouse TNBS-induced model of chronic intestinal inflammation contains several features that are consistent with those observed in Crohn's disease in humans: a chronic colitis is induced that is characterized by a severe, transmural and granulomatous inflammation with infiltrates of macrophages and T cells; there are similarities at the T cell cytokine level since LP $CD4^+$ T lymphocytes in both diseases secrete high amounts of Th1 cytokines such as IFN-γ, but low levels of Th2 cytokines such as IL-4; there is an activation of macrophages with increased production of pro-inflammatory cytokines such as IL-1, IL-6; and finally, we have shown here that LP macrophages in TNBS-induced colitis produce high levels of the pro-inflammatory cytokine TNF-α, consistent with the increased TNF-α levels previously reported in patients with Crohn's disease.

The data reported here provide direct evidence for a predominant role of TNF-α in the mouse TNBS model of chronic intestinal inflammation, and our data strongly suggest that activation of TNF-α is important to maintain chronic experimental colitis. However, TNF-α may not only be important in the pathogenesis of TNBS-induced colitis, but also in other colitis models. For instance, colitis in $IL\text{-}10^{-/-}$ mice has been shown to be associated with a strikingly increased TNF-α production by splenic T cells [25]. Furthermore, antibodies to TNF were successfully used in the CD45 transfer model, in which the adoptive transfer of $CD45RB^{hi}$ T cells from BALB/c mice to C.B.-17 *scid* mice causes chronic intestinal inflammation [28]. In this model, continuous neutralization of TNF-α led to a clear improvement of histologic parameters of established colitis. Taken together, the above data encourage further clinical trials with antibodies to TNF-α for the treatment of patients with Crohn's disease.

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